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**Courtship and Mating Behavior of *Eucelatoria bryani*  
(Diptera: Tachinidae), a Larval Parasitoid of  
*Heliothis* Species (Lepidoptera: Noctuidae)**

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**ABSTRACT** The courtship and mating behavior of *Eucelatoria bryani* (Sabrosky), a larval parasitoid of *Heliothis* spp. and a potentially significant biological control agent, was examined. This laboratory study is the first quantitative study of courtship and mating behavior in the Tachinidae. A stereotypical sequence of behaviors was evident, although the number of behavioral repetitions was variable. Courtship began when a male flew and grasped a walking or flying female from behind. The remainder of courtship following this pair flight occurred on the substrate. The male courted while standing on the female dorsum and copulated with his body positioned behind that of the female. The most conspicuous part of courtship was the male waving his forelegs. Females rejected some copulatory attempts by bucking, although in all cases where a male grasped and held a female, copulation eventually occurred. Mating success depended on the ability of the male to grasp and hold the female.

**KEY WORDS** Insecta, *Eucelatoria bryani*, mating behavior, *Heliothis*

LITTLE QUANTITATIVE INFORMATION is available on the mating behavior of the Tachinidae. Many species of tachinids use aggregation sites for mating and can be categorized according to whether males sit and wait for females or actively search for them (Wood 1987). In species where the male has larger eyes and a narrower frons than the female, males apparently sit and wait for females. In species where the male has eyes similar in size to those of the female but longer antennae, males apparently actively search for females. Based on these morphological characters, males of *Eucelatoria bryani* Sabrosky, which have larger eyes than females, should employ the sit-and-wait strategy. However, an understanding of the behavioral processes involved in courtship and mating is necessary before general hypotheses on mating systems can be developed and tested.

Tachinids are potentially significant biological control agents that have been grossly underexploited, relative to various Hymenoptera. *E. bryani* is one of the most common parasitoids of *Heliothis* spp. larvae in the southwestern United States (Bryan et al. 1972). The practicality of rearing *E. bryani* on a large scale for mass release is currently under investigation (Nettles et al. 1980, Nettles 1987). The mating behavior of any parasitoid being considered as a biological control agent must be understood to monitor the quality of laboratory populations and to ensure that released parasitoids are able to function efficiently in the field (Boller 1972, Huettel 1976).

Our laboratory study of the mating behavior of *E. bryani* is the first quantitative study of mating behavior in the Tachinidae. We sought to deter-

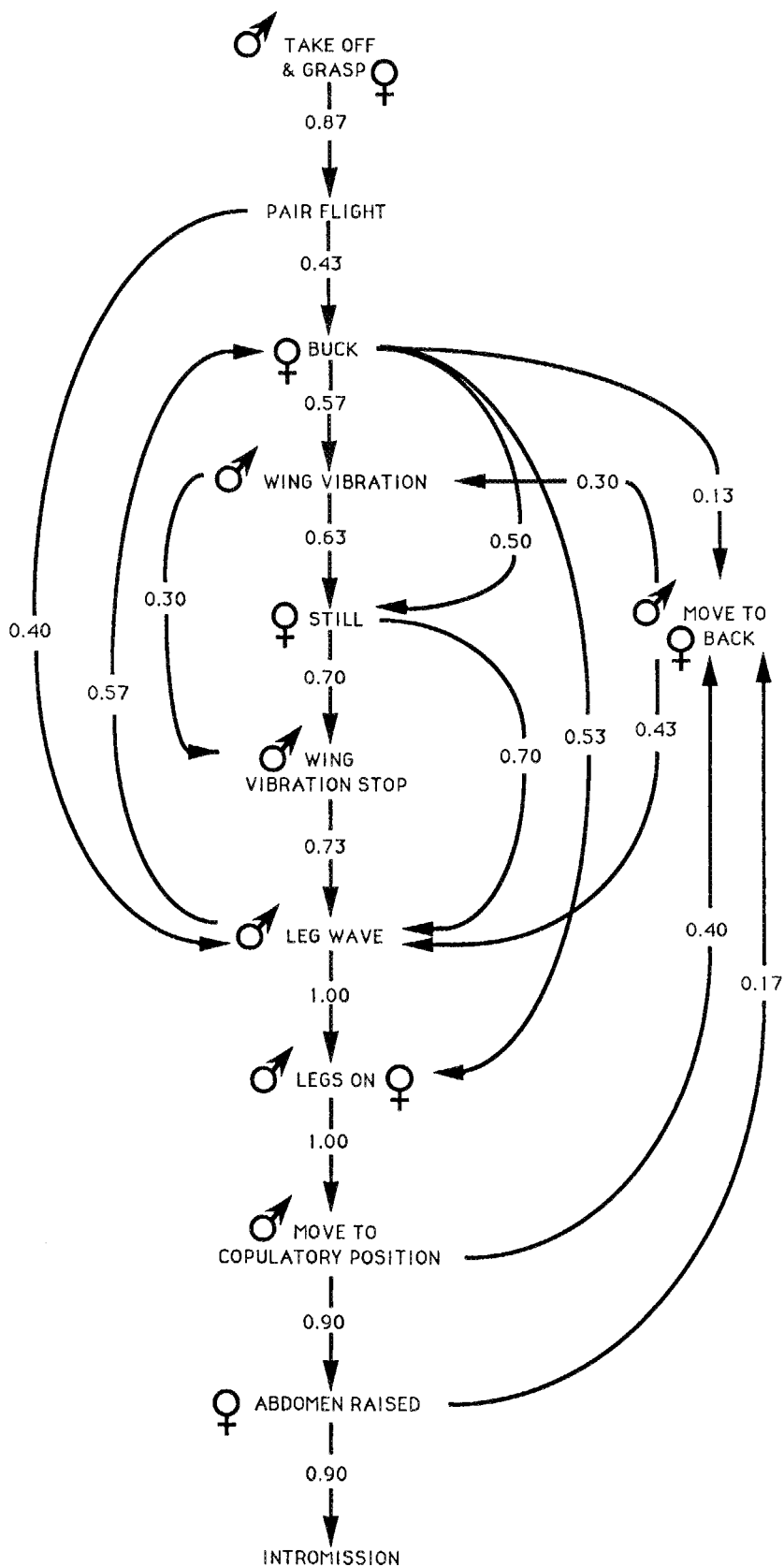
mine the behavioral sequences involved in the courtship of *E. bryani*, variability in courtship and copulation among pairs, and behavioral differences between pairs that mate and those that do not.

#### Materials and Methods

*Eucelatoria bryani* was obtained from a colony established with material collected by C. G. Jackson from corn in Arizona and reared on *Heliothis zea* (Boddie) for the past 5 yr at USDA laboratories in College Station and Weslaco, Tex. We reared *E. bryani* from *H. zea* and *H. virescens* (F.) at Clemson University for 1 yr before testing began. Flies from five separate generations were used in this study. *Heliothis* host larvae were reared individually in 31-ml plastic cups filled halfway with the pinto bean-wheat germ diet of Burton (1969) modified as described by Adler & Adler (1988).

In laboratory colonies of *E. bryani*, male emergence begins approximately 1 d before female emergence, but there is overlap in time of emergence between the sexes. Mating normally occurs within 1 d of female emergence. Therefore, we used adults 1-2 d old for these mating trials.

Two virgin *E. bryani* males were introduced into a Plexiglas arena (15 by 10 by 10 cm) in an environmental chamber maintained at  $26 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH, and a 14:10 (L:D) photoperiod (fluorescent lighting). After 5 min, a single virgin female was introduced. Two males were used to increase the probability of a successful mating and to observe possible male-male interactions. The trios remained together for 20 min (if no mating occurred) or for 5 min after a mating was completed.



Thirty successful and 23 unsuccessful mating trials were recorded with a Panasonic WV-D5000 video camera and later analyzed in both normal and slow motion.

We considered courtship to begin when a male grasped and held a female and to end with intro-mission. Courtship behaviors for successful mating sequences were transcribed into a first-order preceding-following transition matrix. We limited sequences to a single grasp and copulation and did not include self-transitions (i.e., consecutive repetitions of the same behavior). Expected values and standard normal deviates for individual transitions were calculated. The standard normal deviates were analyzed with a binomial test to determine which transitions occurred at a frequency greater than that expected by chance (Bishop et al. 1975, Fagen & Young 1978). Significant transitions ( $P < 0.05$ ) were included in an ethogram showing the most probable sequences of acts in a successful mating. Descriptive statistics were calculated for various aspects of courtship and copulation, including durations (timed to the nearest second) and the number of repetitions of behaviors. Means are reported with their standard errors.

Voucher specimens of *E. bryani* and representative videotapes have been deposited in the Clemson University Arthropod Collection, Clemson, S.C.

### Results

Twelve discrete behavioral acts were identified for the mating behavior of *E. bryani* (see Fig. 1).

**Male Take Off and Grasp.** Once a male was within 3 cm of a female, he flew from behind the female and, while in flight, grasped her.

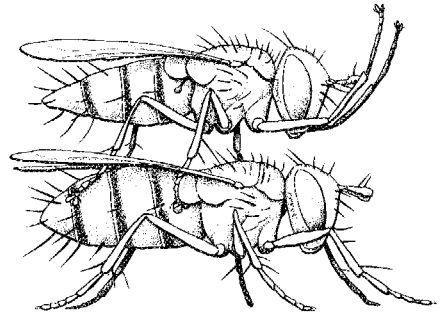
**Pair Flight.** After the male grasped the female, the pair flew in an apparently random path around the arena and then landed.

**Male Wing Vibration.** The male, while grasping the female, vibrated his wings rapidly without initiating locomotion.

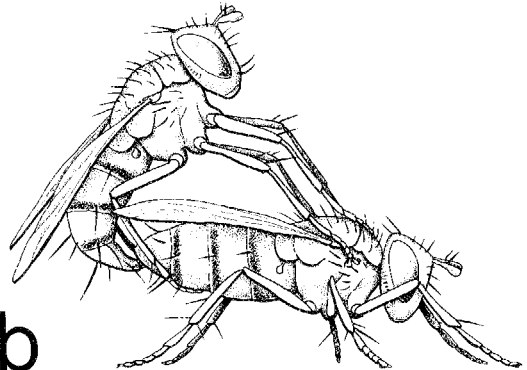
**Male Wing Vibration Stop.** The male ceased vibrating his wings.

**Male Foreleg Wave.** The male raised and lowered his forelegs (Fig. 2a). The upstroke of the leg wave was a smooth motion, with the male raising both legs simultaneously, whereas the downstroke was a rhythmic drumming motion, with the male alternately lowering each leg part of the way down. At the end of the downstroke, the legs of the male were held above, but not in contact with, the eyes of the female.

**Male Forelegs on Female.** Following a foreleg wave, the male placed his forelegs on the dorsum of the female, typically on the anterior scutal region or on the head. This was considered a separate



a



b

Fig. 2. Courtship behaviors of *E. bryani*. (a) Male performing foreleg wave. (b) Pair in copulatory position, with female abdomen raised.

act because it did not occur after each foreleg wave, but after a foreleg wave, the male placed his forelegs on the female before proceeding to a different behavior.

**Male Move to Copulatory Position.** The male moved from standing on the dorsum of the female to a position behind the female (Fig. 2b). While in the copulatory position, the male extended his genitalia to attempt to copulate with the female.

**Male Move to Female's Dorsum.** The male shifted from the copulatory position to his initial position on the dorsum of the female.

**Female Buck.** The female vigorously raised and lowered her body, apparently in an attempt to dislodge the male. In some of the more intense bucks, the female kicked her hind legs and moved about the arena.

**Female Still.** The female became quiescent following a bout of activity.

**Female Abdomen Raised.** The abdomen of the female was raised above the substrate as the male moved to the copulatory position.

Fig. 1. Ethogram of successful matings for *E. bryani*, showing significant behavioral transitions ( $P < 0.05$ ). Values indicate the frequency of the 30 pairs showing a particular transition.

Table 1. First-order behavioral transition matrix for successful matings of *E. bryani* ( $n = 30$ )

Preceding behavior	Following behavior											Σ
	Pair flight	♀ buck	♂ wing vibration	♀ still	♂ wing vibration stop	♂ foreleg wave	♂ forelegs on ♀	♂ move to copulatory position	♂ move to ♀ dorsum	♀ abdomen raised	Intro-mission	
♂ take off and grasp ♀	26 <sup>a</sup> 2.09 <sup>b</sup> 16.5 <sup>c</sup>	4 4.08 —	0 — —	0 — —	0 — —	0 — —	0 — —	0 — —	0 — —	0 — —	0 — —	30 — —
Pair flight	— — —	13 4.74 3.80*	5 2.91 1.23	1 3.99 —	0 — —	12 5.82 2.56*	0 — —	0 — —	0 — —	0 — —	0 — —	31 — —
♀ buck	2 4.48 —	— — —	17 6.01 4.48*	15 8.25 2.35*	0 — —	0 — —	16 6.57 3.68*	0 — —	10 4.20 2.83*	0 — —	0 — —	60 — —
♂ wing vibration	3 2.54 —	3 5.56 —	— — —	19 4.69 6.61*	9 2.46 4.16*	2 6.83 —	0 — —	0 — —	0 — —	0 — —	0 — —	36 — —
♀ still	0 — —	4 7.80 —	0 — —	— — —	21 3.45 9.44*	21 9.58 3.69*	1 5.23 —	2 3.68 —	0 — —	0 — —	0 — —	49 — —
♂ wing vibration stop	0 — —	4 5.07 —	0 — —	6 4.27 0.84	— — —	22 6.22 6.32*	0 — —	0 — —	1 2.17 —	0 — —	0 — —	33 — —
♂ foreleg wave	0 — —	17 7.97 3.20*	0 — —	2 6.72 —	1 3.53 —	— — —	30 5.35 10.65*	0 — —	0 — —	0 — —	0 — —	50 — —
♂ forelegs on ♀	0 — —	8 13.28 —	12 8.16 1.34	11 11.20 —	0 — —	14 16.32 —	— — —	30 6.26 9.48*	2 5.69 —	1 5.31 —	0 — —	78 — —
♂ move to copulatory position	0 — —	12 8.87 1.05	0 — —	0 — —	0 — —	1 10.90 —	0 — —	— — —	12 3.80 4.20*	27 3.55 12.45*	3 3.80 —	55 — —
♂ move to ♀ dorsum	1 2.24 —	3 4.90 —	9 3.01 3.45*	5 4.13 0.43	0 — —	13 6.02 2.84*	0 — —	1 2.31 —	— — —	0 — —	0 — —	32 — —
♀ abdomen raised	0 — —	2 5.40 —	0 — —	0 — —	0 — —	1 6.63 —	0 — —	0 — —	5 2.31 1.77*	— — —	27 2.31 16.2*	35 — —
Σ	32	70	43	59	31	86	47	33	28	30	30	459

\*, transition that occurred at a frequency greater than that expected by chance.

<sup>a</sup> Observed number of mating pairs showing transition.

<sup>b</sup> Expected number of mating pairs ( $E = r \cdot c / N$ ), where  $E$  is the expected value for transition  $i$ ,  $r$  is the row total for transition  $i$ ,  $c$  is the column total for transition  $i$ , and  $N$  is the grand total for all transitions.

<sup>c</sup> Standard normal deviate ( $Z$ ).

**Intromission.** The male successfully copulated with the female.

Twenty-one of the possible behavioral transitions were performed by a significant number of the mating pairs (Table 1). A common trend in the sequence of these behaviors was evident (Fig. 1), although the repetition of behaviors among mating pairs was variable.

Female movement appeared to be an important cue for initiating male activity. In 87% of the successful trials ( $n = 30$ ), the female was moving (walking or flying) immediately before a male grasped and held her securely. In 20 of the trials, female movement appeared to be nondirectional, but in 6 trials the female was moving directly toward the male.

Once a male grasped and held a virgin female, the pair proceeded through courtship and copu-

lated (100%,  $n = 30$ ). Males were not always successful in grasping and holding a female. In the trials where mating did occur, males that did mate grasped the female  $1.4 \pm 0.35$  times, including the final successful attempt. The unsuccessful males in the trials where mating did occur grasped the female significantly fewer times ( $0.2 \pm 0.10$ ) ( $t = 3.11$ ;  $df = 23$ ;  $P = 0.0049$ ; incomplete data for six trials). The four unsuccessful males that did grasp the female always separated from the female during flight.

In 23 trials, no mating occurred. In four of these trials, the female remained still and no intersexual interactions occurred. In 14 of the trials, males pursued the female but were unable to grasp her. In the other five of these trials, males were able to grasp the female, but the pairs separated during flight.

**Table 2.** Duration of phases of successful matings for *E. bryani* in the laboratory at  $26 \pm 1^\circ\text{C}$ 

	n	Duration, min			CV
		$\bar{x}$	SEM	Min-max	
Female introduction into arena until courtship initiation	29	7.27	1.24	0.55-23.28	92.13
Courtship	30	1.77	0.47	0.25-11.98	146.38
Copulation	30	11.00	1.61	5.92-54.45	80.11

When not in courtship or copulation, males oriented head down on the sides of the arena or flew and walked about the arena, particularly during chases involving either the female or the other male. In 60% of the trials where mating occurred ( $n = 30$ ), males grasped the other male and, in three of these cases, they initiated courtship before being dislodged by bucking of the other male.

The most conspicuous aspect of courtship was the male foreleg wave which the male performed in all successful trials. Males performed an average of  $12.9 \pm 3.06$  foreleg waves during a courtship, with at least two foreleg waves occurring before the final successful copulatory attempt. This last bout of leg-waving occurred within 3 s of the male moving to the copulatory position and without the female bucking. When the male moved to and remained in the copulatory position, his forelegs appeared to grasp setae on the anterior scutal region of the female.

During courtship, the male moved to the copulatory position and attempted to copulate with the female an average of  $3.4 \pm 0.71$  times, including the final successful attempt. While in the copulatory position, the male moved his abdomen back and forth horizontally in attempts to copulate with the female. A male was unsuccessful in copulating when he could not grasp the female genitalia while her abdomen was raised (12 trials) or when the female did not have her abdomen raised (5 trials). After each unsuccessful copulatory attempt, the male returned to the female dorsum and repeated his courtship display.

Bucking appeared to be an attempt by the female to dislodge the male because it occurred at specific points during courtship, but it did not occur in all trials. The intensity of bucking ranged from rapid raising and lowering of the abdomen, to kicking the male with the hind legs, to moving around the arena. A male normally responded to female bucking by vibrating his wings (17 trials), following which the female became quiescent. However, the female frequently became still without the male vibrating his wings (15 trials).

As a result of differences in the number of times acts were repeated, the length of courtship was highly variable; however, copulation times also were highly variable (Table 2). While in copula, the pairs remained still. In two trials, the second male interfered with the courting pair by mounting the first male and initiating his own courtship. This

interference lasted  $<4$  s before the second male dismounted. In these trials, the second male was not able to dislodge the first male, and the original pair continued to court in a normal manner.

We did not observe any behavior on the part of the female that signaled the end of copulation. Pairs separated when the male pushed off the female with his forelegs, turned, and moved away. Following copulation, the female usually remained stationary and groomed her terminalia.

## Discussion

The behavior of *E. bryani* in this study lends credence to the morphological evidence that males of this species sit and wait for potential mates to move past them (Wood 1987). Males in our study generally rested head down on the sides of the arena, flying out occasionally in pursuit of other individuals, both male and female. The fact that males would chase, grasp, and occasionally begin to court other males (as well as pairs in courtship) indicates that they may not be able to discriminate sexes immediately, a situation demonstrated in other Diptera (Tobin & Stoffolano 1973a,b; Speith 1974; Scarbrough 1981).

The pair flight might serve an indirect, dual function. Instead of landing directly on the substrate, the pair moved in an apparently random pattern around the arena. Although this movement might be only the result of struggles between the male and female and of the confines of the arena, an erratic path in the field might move the pair away from an aggregation site, decreasing the probability of other males interfering and of predators following and locating the pair. In the laboratory, we have observed mating pairs being mounted by up to three males at one time, although in none of these instances was the original male displaced.

The ability of a male to grasp and hold a female successfully was the most important element in determining his mating success. Once a male succeeded in grasping and holding a female, courtship behavior was fairly stereotypical and always proceeded until copulation was completed. The foreleg wave appears to be another important element of courtship because males performed it in all courtships and at least two times before moving to the copulatory position for the final time. But exactly how the male foreleg wave functions in courtship is unclear. Because the forelegs of the male are held out over the eyes of the female, the foreleg wave might serve as a visual cue. Males of *Calliphora vomitoria* (L.) (Diptera: Calliphoridae), while mounted on the female dorsum, "tread" their forelegs over the anterior thorax and eyes of the female, providing visual or tactile stimuli (Campan & Lauga 1981). In response, the female remains still or indicates her unreceptiveness by flying or walking. *Physiphora demandata* (F.) (Diptera: Otitidae) employs a display similar to that of *E.*

*bryani*. Before mounting a female, males of *P. demandata* wave one metathoracic leg while facing the female. This movement, which has been interpreted as a visual signal, and other displays are repeated until the female signals her receptiveness and the male mounts her (Alcock & Pyle 1979).

*Eucelatoria bryani* females appeared to signal their unreceptiveness by bucking or not raising the abdomen. Female bucking interrupted any male behavior and forced the male to respond in a specific manner. Active attempts by females to dislodge a courting male are common among Diptera (e.g., Roth 1948; Tobin & Stoffolano 1973a,b; Campan & Lauga 1981; Scarbrough 1981). The female of *E. bryani* also can reject copulatory attempts by not raising her abdomen. Raising the abdomen is probably necessary for the female to expose her genitalia to those of the male (Tobin & Stoffolano 1973a).

The most common male response to female bucking was the wing vibration, which appeared to quiet the female by an unknown mechanism. Wing vibrations might create mechanical or auditory stimuli that inhibit female movement (Mathews 1975). Speith (1952) suggested that wing vibrations by male *Drosophila* may serve as auditory stimuli to females or may disperse odors to the female, but as yet the Tachinidae have not been studied for the presence of pheromones. Wing vibrations are an important signal in the courtship of *Musca domestica* L. and *M. autumnalis* De-Geer, where the male vibrates his wings from the time he mounts the female until he moves to the copulatory position (Tobin & Stoffolano 1973a,b). However, Campan & Lauga (1981) interpreted wing vibrations of male *C. vomitoria* as a means to maintain balance.

The courtship behaviors of *E. bryani* do not have an easily explained intraspecific role because once a male grasped and held a female, neither refused to mate with the other. This lack of discrimination is interesting because *E. bryani* females appear to mate only once. Therefore, a larger role in mate selection may need to be ascribed to behaviors that occur before males and females make contact. Possibly just the ability to grasp a female is an adequate indicator to the female of the fitness of a male, or persistence in attempting to grasp the female may influence male mating success. Alternatively, courtship in *E. bryani* might function, in part, as a reproductive isolating mechanism.

The length of courtship in *E. bryani* and the reason that males need to perform acts several times following unsuccessful courtship attempts are still unexplained. The repetition of courtship behaviors might result from courting males testing female receptivity by attempting copulation without receiving any signal from the female (Alcock & Pyle 1979). *Drosophila* males usually make more than one copulatory attempt because females are rarely receptive after the first courtship bout (Speith 1974). Testing female receptivity might be advantageous

to the male by minimizing the time spent in courtship and would account for the repetition of behaviors and unsuccessful copulatory attempts that we observed in *E. bryani*. Because the female must raise her abdomen for copulation to take place, the female would seem to control when copulation takes place, although perhaps she cannot control with whom she mates. Therefore, the role of the rather complex courtship of *E. bryani* might be to induce complete receptiveness on the part of the female.

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